

LEWIS CAMERON PRIZE

INFECTIOUS MONONUCLEOSIS

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May, 1970.

Introduction

Infectious mononucleosis is a common, benign illness of adolescents and young adults, which, at first glance, seems rather uninteresting. However, a brief examination of the blood shows that there is an outpouring of atypical mononuclear cells. The immunoglobulin levels are raised and the serum contains a variety of heterophil and auto-antibodies as in many of the immuno-proliferative and lympho-reticular disorders.

Until recently no causative agent had been found but two years ago a connection was found with the EB virus which had previously been detected in many cases of Burkitt's lymphoma, a tumour, mainly of the jaw, affecting young children in Africa and New Guinea.

Thus study of this disease may help to solve some of the problems of the acute leukaemias, the malignant reticuloses and possibly of auto-allergy. Further investigation of the role of the EB virus may show how certain viruses can alter the normal allergic response and what the connection is between infectious mononucleosis and Burkitt's lymphoma.

Early History of Infectious Mononucleosis

This account is taken from Carter and Penman (1969a).

Pfeiffer's Drüsenfieber

This term was introduced by Pfeiffer in Germany in 1889 but it seems probable that this, or a very similar, condition had/

had been described in Russia before 1889. Drüsenfieber was a common benign fever in young children. It was sudden in onset and usually of short duration but occasionally lasted for a week or so. It was characterised by malaise, fever, sore throat and cervical lymphadenopathy.

Pfeiffer also noticed some ill-defined chronic cases of glandular fever which was apparently infectious especially in families (Hausepidemien). Pfeiffer thought that Drüsenfieber comprised a number of different pathological conditions but this point was ignored by his contemporaries so that Drüsenfieber was accepted as a new disease entity.

After this several accounts of it were published but it became confused with lymphadenitis secondary to local infection. Partly because of this and partly on clinical grounds alone it is now impossible to characterise.

In 1921 Tidy and Morley revived interest in glandular fever with a review of the haematology and a report on a case. Lymphocytosis was inconstant and abnormal leucocytes were not often seen.

Infectious Mononucleosis

In 1920 Sprunt and Evans published an account of 5 sporadic cases in young adults of "infectious mononucleosis" characterised by a gradual onset, fever and lymphadenopathy. Pharyngitis and splenomegaly were also noted. The fever lasted 2 - 3 weeks but recovery was complete. Haematological examination showed a mononuclear/

Table 1.

Frequency of symptoms and signs in infectious mononucleosis

<u>Symptom or sign.</u>	<u>Frequency.</u>
Lymphadenopathy	90 - 100
Fever > 100°F.	75 - 95
Pharyngitis	70 - 85
Splenomegaly	25 - 60
Eyelid oedema	12 - 40
Belated exanthem	25 - 40
Hepatomegaly	5 - 20
Jaundice	5 - 15
Rash	3 - 15

Data from Hobson, Lawson and Wigfield 1958

Evans 1960

Hoagland 1960a

Dunnet 1963

Joncas, Chiasson and Turcotte 1968

Finch 1969a

mononuclear leucocytosis with large numbers of abnormal pleomorphic mononuclear cells present. Before this there had been a few reports of similar isolated cases, often misdiagnosed as acute leukaemia.

The situation then became very confused since Drüsenfieber and infectious mononucleosis were considered as one condition although they are obviously two separate diseases.

By 1926 it was becoming apparent that the haematological changes in infectious mononucleosis (IM) were not specific. It is now known that the abnormal cells can occur in many different conditions. Not until the development of the Paul-Bunnell-Davidsohn test was it possible to define IM with certainty. Now IM is diagnosed on the basis of the clinical, haematological and serological findings (Hoagland 1960b).

Clinical Features and Laboratory Findings

Symptoms and Signs (See Table 1)

This account is drawn from Hobson, Lawson and Wigfield (1958), Evans (1960), Hoagland (1960a), Dunnet (1963), Joncas, Chiasson and Turcotte (1968) and Finch (1969a).

The onset of IM is usually insidious and the early symptoms very non-specific, the main ones being malaise, anorexia and headache.

The most important symptom is the sore throat which develops a few days after the onset of symptoms. It usually increases in severity/

Table 2.

Frequency of clinical varieties of infectious mononucleosis

<u>Type</u>	<u>Incidence</u>
Pharyngeal	80%
Typhoidal or essential (no pharyngitis or jaundice)	12%
Icteric	8%

severity for 3 - 6 days and then rapidly subsides. It is only relieved by steroids. Sometimes it is severe enough to interfere with swallowing.

Lymphadenopathy occurs in almost all patients and is usually detectable by the end of the first week. The swelling has usually subsided after 4 weeks. The enlargement is symmetrical and moderate especially in the posterior cervical region. There is usually axillary and inguinal lymphadenopathy. Hilar enlargement is rare (in 3 out of 200 cases in one survey (Hoagland 1960a)).

Pharyngitis is also seen in most cases and follows the same course as the sore throat. Hyperplasia of the pharyngeal lymphoid tissue is almost always present. About half the cases have an exudate.

Other, less important symptoms and signs are listed in Table 1, together with their incidence. The incidences given by Hobson et al (1958) are lower than those in other reports, possibly because their cases were drawn from the general population and so with a milder illness than those in hospital.

Hoagland (1960a) has identified 3 types of IM based on the differing clinical features (See Table 2).

Course

In general, the illness lasts as long as the fever which is usually about 2 weeks (Hoagland 1960a). The shorter the illness the quicker the return to normal but lethargy and malaise can persist/

persist for weeks or months (Dunnet 1963). The disease can be very mild, the patient remaining active although feeling a bit unwell (Bender 1958, Finch 1969a). Children are especially liable to have it mildly, IM not being suspected. However, adults over 30 may have a severe attack.

Relapses occasionally occur after 2 - 3 afebrile days but Hoagland (1960a) never saw one after 5 days. Anamnestic heterophil antibody response to mild respiratory infection may be responsible for some of the reports of late relapse (Bender 1958).

Complications

These are fortunately very rare although the mortality from them is appreciable. Only the more interesting are mentioned here. Others are discussed by Dunnet (1963) and Finch (1969a).

Rarely, pharyngitis may be severe enough to cause almost complete pharyngeal obstruction but this can be relieved by steroids (Finch 1969a).

Acquired haemolytic anaemia, probably auto-allergic in origin, is an interesting complication, the pathogenesis of which is discussed later. Thrombocytopenic purpura is rare although slight thrombocytopenia is common (Carter 1965a); this also is discussed later.

Laboratory Findings

Haematology/

Haematology. The total white cell count is usually normal or slightly raised and high counts are rare (Bender 1958, Finch 1969b). There may occasionally be a leucopenia in the first few days (Dunnet 1963).

The leucocytosis is mainly due to the absolute and relative increase in the numbers of normal lymphocytes and the pleomorphic atypical mononuclear cells (AMCs). Various attempts to classify the latter have been made but none are really satisfactory.

The AMC is not pathognomonic of IM since it is found in normal adults (up to 15% of mononuclear cells) and in a number of infections and drug reactions. In IM the percentage of AMCs is always ^{at least} 20% (Bender 1958) which Wood and Frenkel (1967) defined as the lower limit for a definite atypical lymphocytosis. Counts up to 80% have been recorded (Dunnet 1963).

There is a relative and absolute neutropenia in most cases with a shift to the left (Bender 1958, Carter 1966a). Anaemia is rare but thrombocytopenia is common (Carter 1965a).

Serology. In IM a heterophil antibody appears which can be differentiated from the Forssman antibody. It agglutinates sheep red cells, is not absorbed by guinea pig kidney but is absorbed by ox red cells. This is the Paul-Bunnell (PB) antibody and the test the Paul-Bunnell-Davidsohn (PBD) test. Davidsohn (Davidsohn and Lee 1969) considers any residual agglutinin activity left after guinea pig kidney absorption as positive provided the absorption pattern is typical, although some workers have set a/

a lower limit such as Bender (1958) and Hoagland (1960a).

The titre rises to a maximum in the third and fourth weeks (Dunnet 1963) by which time most cases have a positive PBD test (Hobson et al 1958). The test reverts to negative after 8 - 12 weeks (Finch 1969b) but the antibody can occasionally persist for long periods (Hobson et al 1958). Some cases remain PBD negative although on clinical and haematological grounds they are typical cases of IM. These seronegative cases are considered below.

Epstein-Barr virus (EBV) antibodies have been detected in IM sera (Henle, Henle and Diehl 1968) but their place in routine diagnosis is uncertain at the moment.

Liver Function. Most cases have evidence of mild hepatitis (Evans 1960, Dunnet 1963) although overt jaundice is rare. The most sensitive indicator of liver damage is isocitric dehydrogenase (Dunnet 1963); SGOT was also raised but in fewer cases. The enzyme levels returned to normal in 6 weeks. Dunnet (1963) found that liver function was often normal in the presence of greatly increased serum enzyme levels although Evans (1960) and Joncas et al (1968) found some degree of abnormal function in most cases. Serum bilirubin is slightly raised in about one third of cases (Dunnet 1963).

Seronegative Infectious Mononucleosis

A case of seronegative IM could be due to one of a number of causes. The following list of possibilities is from Penman (1969):/

(1969):

Listeriosis	}	Not important in U.K. and U.S.A.
Infections with Rickettsia Sennetsu		
Acquired toxoplasmosis		
Cytomegalovirus infection		
Drug reactions		
Blood transfusion.		

Toxoplasmosis (Penman 1969). Infection is usually symptomless but when symptoms do occur they are similar to those of IM. The PBD test is said to be negative. Diagnosis is by demonstrating a high or rising antibody titre. In Britain it is probably a rare cause of seronegative disease.

Cytomegalovirus infection. Klemola and Kaariainen (1965) first described seronegative IM with rising titres to cytomegalovirus (CMV). Stern (1968) demonstrated CMV infection in young children and adults over 30 but not in those in the 15 - 29 age group. Joncas et al (1968) have also suggested that CMV and adenoviruses are possible causes of seronegative IM. CMV may be responsible for a large proportion of seronegative cases especially in children but serological diagnosis is difficult since sub-clinical infection is common (Penman 1969).

Blood transfusion. An IM-like syndrome following cardio-pulmonary bypass has been reported (e.g. Riemenschneider and Moss 1966). Heterophil antibody was found in 2 cases but the full PBD test was not done. In all the reports blood transfusion is/

is the only common factor so the infection is presumably blood-borne.

Embil, Folkins, Haldane and van Rooyen (1968) and Lang, Scolnick and Willerson (1968) found evidence of CMV infection in some cases after perfusion. Some were symptomless but others had the post-perfusion syndrome. In the group studied by Embil et al (1968) two cases had a positive PBD test, one of whom was symptomless. This could be a case of subclinical IM. However, CMV is not responsible for all cases of this syndrome. Gerber, Walsh, Rosenblum and Purcell (1969) found evidence of EBV infection in 5 cases after cardio-pulmonary bypass. One developed an IM-like illness with PB antibody in the blood. Her buffy-coat cells were established in culture and shown to contain EBV antigen. All these cases were free of EBV antibodies pre-operatively. This shows that EBV infection can be sub-clinical and that the virus can cause an illness virtually identical with IM.

Various workers have pointed out the difference between seronegative and seropositive IM, e.g. Hobson et al (1958), Evans (1960), Belfrage (1962). The seronegative disease was milder and tended to occur in children and older adults. It was thought to be a separate disease although there were one or two cases developing in contacts of seropositive cases.

Recently, it has been found that some seronegative cases develop EBV antibodies (Evans, Niederman and McCollum (1968), Gerber/

Table 3.

Incidence of infectious mononucleosis in various communities

<u>Prop.confirmed by lab.</u>	<u>Place</u>	<u>Period</u>	<u>Av.annual incidence</u> <u>/10,000</u>
Not known	{ Notifications: Bristol ¹	1963-6	3.1
	{ " : N.Ireland ¹	1959-66	0.5
	{ Reports: family doctors ¹	1955-6	6.0
	{ Reports: " ¹	1966-7	2.4
All	Survey: Oxford ²	1954-6	5.6
All	" : Portsmouth area ³	1962-3	3.8
	Malmö ⁴		3 - 3.5
	Connecticut ⁵	1968	4.5

1. From Pollock 1969.

2. Hobson et al 1958.

3. Penman 1966.

4. Belfrage 1962.

5. Evans et al 1968.

Gerber, Hamre, Moy and Rosenblum (1968) but the role of the EB virus in IM is not yet clear. However, IM and similar conditions may be tentatively grouped into 3 categories (Penman 1969):

1. Patients with both EBV and heterophil antibodies: the traditional seropositive IM.
2. EBV positive and PBD negative: some cases of the seronegative group.
3. EBV negative, PBD negative: the rest of the seronegative group with a variety of conditions.

Epidemiology

Incidence

The incidence of IM in the general population is difficult to determine since it is not notifiable. From Table 3 it can be seen that the incidence does not vary greatly and is about the same in America, Sweden and Britain. Newell (1957) found a much lower frequency which tended to increase from north to south (0.2 - 0.59/10,000). This regional difference probably reflects differences in the use of laboratory services. The lower incidence suggests that only about one tenth of cases are confirmed serologically. In college communities the incidence is very much higher at 30 - 150/10,000 (Evans et al 1968).

IM tends to attack young people in the 15 - 24 age group and is relatively uncommon in younger and older people (Newell 1957, Hobson et al 1958, Belfrage 1962, Dunnet 1963, Ødegaard 1967/

1967, Joncas et al 1968).

Young children rather than young adults tend to be more susceptible to disease to which susceptibility varies with age. This means that young adults are either more susceptible or more exposed to infection (Pollock 1969). Evans (1960) has suggested that IM might occur in an unrecognised form in children and only becomes manifest later.

Overall there is an equal sex incidence (Newell 1957, Hobson et al 1958, Evans 1960, Ødegaard 1967) although affected males tend to be older than affected females so more females are in the 10 - 19 age group (Hobson et al 1958, Ødegaard 1967).

There does not appear to be any definite seasonal variation in the incidence of IM although it tends to be commoner in the spring (Newell 1957, Dunnet 1963, Hobson et al 1958). Hoagland (1964) found that in army cadets the incidence was greatest just after their vacations.

Transmission

IM is not particularly infectious although it can occasionally occur in contacts (Hobson et al 1958, Evans 1960, Hoagland 1964) although Hoagland (1964) has not seen IM developing in room-mates of cases.

The method of transmission is not yet known with certainty. Kissing and shared drinking vessels have both been suggested as possible ways (Hoagland 1955, 1964), the common factor being the exchange of saliva. Evans (1960) confirmed that,

that kissing was a possible mode of transmission but suggested that other social factors associated with kissing may be more important. Ødegaard (1967) provides some support for a sexual basis for transmission by pointing out the similarity in the age distribution of IM and gonorrhoea, the peak for gonorrhoea being 2 years later. A disease transmitted by kissing would tend to occur in younger people than one spread by sexual intercourse (Pollock 1969).

Hoagland (1955) suggested that chronic carriers were the source of infection since very few cases had a contact history. Evans (1960) in confirming this found that only 25% of contacts had had the disease.

Incubation Period

After reviewing the literature and taking into account his own findings Evans (1960) thought the incubation period to be 7 - 50 days. Hoagland (1964) and Joncas et al (1968) narrowed it down to 34 - 49 days by considering the contact histories. The very short periods could be due to 2 cases acquiring IM from the same source and developing it a few days apart.

Subclinical Disease

There is no direct evidence for the existence of sub-clinical disease except possibly in one outbreak (Watson, Johnson, Kahn and Stone 1951). However, some cases may be very mild and cause minimal discomfort so that IM is not diagnosed. Contact surveys are not very helpful in this regard since, because/

Table 4.

Cytochemical Reactions

Positive:	Glycogen	Phosphorylase
	RNA (variable)	Acid phosphatase
	Lactic dehydrogerase	ATP-ase
	Succinic dehydrogerase	Non-specific esterase
	Reactive-SH groups	
Negative:	Alkaline phosphatase	Peroxidase
	"Nodi-oxidase"	
Equivocal:	Glycoproteins	Lipids

From Carter 1966b and

Galbraith, Mitus, Gollerkeri and Dameshek 1966

because of the long incubation period, the source of infection is probably completely free of the disease.

Outbreaks

The last reported major epidemic was in New York in 1951 (Watson et al). Hoagland (1955) reviewed all the reports up to then and considered that in most cases the diagnosis of IM was not justified because of faulty technique in the PBD test or because they occurred before the PB test was developed. In the 1951 epidemic, although the diagnostic criteria were not as rigid as now (see Hoagland 1960b), some cases seemed to have had mild or sub-clinical IM. It seems unlikely, therefore, that IM occurs in large-scale outbreaks.

Haematology

The characteristic haematological feature of IM is the atypical mononuclear cell (AMC).

Morphology

The most striking feature of the AMC is its pleomorphism; it can have lymphocytoid, plasmacytoid or monocytoid features. Many attempts have been made to classify the various types, the latest by Wood and Frenkel (1967), but none are very successful, mainly because there is a continuous spectrum.

Cytochemistry

The cytochemical reactions of the AMC are listed in Table 4. The reactions differ from those of normal monocytes and lymphocytes/

lymphocytes but are only relative and do not justify putting the AMC into a separate category (Carter 1969b). Indeed, the general staining pattern suggests they are predominantly lymphocytic (Carter 1966b). The amount of RNA present is very variable and in a few cells is considerable, indicating active protein synthesis (Carter 1966b).

Ultrastructure

This has been described by Paegle (1961), Inman and Cooper (1965) and Cooper (1969). Despite their pleomorphism, the AMC appears to have a remarkably consistent ultrastructure (Paegle 1961) which is very similar to that of lymphocytes transformed by phytohaemagglutinin (PHA) apart from the lack of rough endoplasmic reticulum (Inman and Cooper 1965, Cooper 1969). Inman and Cooper (1965) also showed that both types of cell have more ribosomes and mitochondria and a more elaborate Golgi apparatus when synthesising DNA. Peripheral leucocytes from IM in long-term culture have also been shown to be very similar to PHA-transformed cells (Glade, Kasel, Moses, Whang-Peng, Hoffman, Kammermeyer and Chessin (1968).

Virus particles have not been seen in fresh preparations of AMCs but have been seen in cells in long-term culture, previously shown to contain EB virus antigens (Diehl, Henle, Henle and Kohn, 1968). Moses, Glade, Kasel, Rosenthal, Hirshaut and Chessin (1968) have seen the EBV particles and also smaller 22 m μ particles in similar cells/

cells.

Antibody Formation

Because of their plasmacytoid features and their protein synthesising activities the AMC has been suggested as the source of the various antibodies produced in IM (Cooper 1969). However, the situation is not yet clear.

Evans (1960), Galbraith et al (1963) and Carter (1966e) found that no or very few cells in the peripheral blood were producing immunoglobulins. Carter (1966e) thought that those that were belonged to the plasma cell series. On the other hand, van Furth, Schuit and Hijmans (1966) observed that a few AMCs were forming IgM and IgG immunoglobulins.

Carter (1966e) failed to detect any antibody formation but Mackinney (1968) claimed that in short-term culture immunoglobulins and even heterophil antibody were produced, the latter detected only by radio-immunoelectrophoresis. PHA was used to stimulate these cultures so that the immunoglobulins may have been produced by transformed lymphocytes and not by AMCs.

Cells in long-term culture could produce IgM, IgG and IgA but no specific antibody (Glade et al 1968b, Glade and Chessin 1968). However, there is some doubt about the relationship between these cells and AMCs (Glade and Chessin 1968).

In lymph-node aspirates, Galbraith et al (1963) found two types of cell: one a commoner, primitive cell very similar to the AMC, showing no antibody-synthesising activity; the other, characterised/

characterised as a haemocytoblast or immunoblast, showed evidence of antibody production.

Carter (1966e) confirmed and extended these findings. In lymph nodes and bone marrow, the number of IgM-producing cells was increased compared with controls but IgG production was not impaired. Some of the cells belonged to the plasma cell series. Others, mainly the IgM producers, were pleomorphic and difficult to identify but were thought to be plasma cell precursors (Carter 1966b, e).

Proliferative Activity

AMCs in mitosis are occasionally seen in ordinary films but are more frequently in white cell concentrates, especially in the first 3 weeks of the illness (Carter 1965b).

Using tritiated thymidine (^3HT), Epstein and Brecher (1965) and Carter (1965b) showed that up to 10% of the AMCs were labelled in the early phase of the disease, the percentage declining thereafter. Prior to this work, Bertino, Simmons and Donohue (1962) showed that there was increased activity of the enzymes involved in folic acid metabolism which coincided with the increased number of cells synthesising DNA in the peripheral blood.

More detailed study of DNA synthesis of peripheral leucocytes has been carried out by MacKinney (1965, 1967) and by Cooper, Hale and Milton (1967).

Measurement of DNA content and ^3HT labelling have shown the cells awaiting mitosis (in G2) form only 1 - 2% of the total AMC population /

population (Cooper et al 1967), possibly because G2 was very short compared with that of PHA-transformed cells and possibly because most cell division occurs outside the blood.

In these short-term cultures some cells stopped synthesising DNA and others accumulated in G2 (MacKinney 1965, Cooper et al 1967) but the relevance of this to in vivo division is doubtful.

Galbraith et al (1963) found that there was very little RNA synthesis in the AMCs in the peripheral blood. In contrast to this, Epstein and Brecher (1965) found that up to 12% of AMCs were synthesising RNA in parallel with DNA synthesis. There is now some evidence to suggest that the rate of RNA synthesis is higher in cells synthesising DNA and in G2 than in cells yet to begin DNA synthesis and in out-of-cycle cells (Topping in Cooper 1967).

PHA appears to depress RNA synthesis in fresh AMCs (Rubin 1966) which suggests that their activity is already maximal when freshly isolated.

Why so few AMCs in the circulation are synthesising DNA or are in G2 is not known for certain. Cooper (1969) has suggested some possibilities: the conditions in the circulation may not be suitable or, more likely, the dividing cells have overflowed from their production site.

Origin and Fate

Despite the vast increase in number of these primitive lymphoid cells, there is no accompanying increase in the numbers of normal lymphocytes and monocytes in the tissues (Carter 1966b).

Carter/

Carter (1966b) has put forward two possible origins for the AMC. He points out that these cells, intermediate in structure between reticulum cells and small lymphocytes, occur in small numbers in normal and hyperplastic lymphoid tissue. One possibility is that their maturation into small lymphocytes is impaired. The second is that there are 2 categories of AMC: one related to normal lymphoid cells, the second derived from abnormal lymphoid cells which might have arisen during the increased lympho-reticular proliferation and which might be very pleomorphic.

In vitro, some of the cells synthesising DNA appear to divide 1 - 3 times and end up as cells resembling small lymphocytes (MacKinney 1965). However, these cells may be large lymphocytes, not AMCs, which are known to develop into small lymphocytes (See Gowans and McGregor 1965). Their fate in vivo is unknown and is difficult to determine.

Comparison with Leukaemic Cells

Dameshek (1969) has suggested IM is a benign self-limiting form of leukaemia. In contrast to leukaemic cells, the AMC is pleomorphic and its DNA synthesis is normal. But, as in leukaemia, there is intense, uncontrolled lympho-reticular proliferation and infiltration of tissues (Carter and Penman 1969b). Why the proliferation ceases so suddenly is unknown.

Granulocytes

As already mentioned, there is a neutropenia and shift to the left in IM. The neutrophils have been shown to be morphologically and/

and cytochemically immature (Carter 1966a).

Despite the neutropenia, which can be reversed if a bacterial infection supervenes, the bone marrow is hyperplastic and there is no evidence of defective maturation or release.

Carter (1966a, 1969a) has tried to explain the neutropenia which must be due to excessive peripheral destruction since maturation and release appear normal. The structural changes in the spleen (Carter and Penman 1969b) and the splenomegaly must lead to pooling.

Leucoagglutinins have been found in some sera and could be involved but their significance is doubtful (Carter 1966d).

Platelets

Carter (1965a) found that platelet levels were reduced below the normal range in about half his cases. Although this slight thrombocytopenia is common, clinical thrombocytopenia purpura is very rare indeed (Sharp 1969).

Again, there is no satisfactory explanation. Sharp (1969) has reviewed the various theories put forward. The more plausible ones are the following. The causative agent could infect the megacaryocytes and inhibit platelet production. Or the virus could alter the antigenic structure of, or act as a buffer on, the platelet so that anti-platelet antibodies would be formed. There is, indeed, some not very convincing evidence for these (Carter 1966b, Sharp 1969). Or again the virus might damage the platelets directly.

Serology/

Serology

Immunoglobulin Levels

Wollheim (1968) has shown that the levels of all classes of immunoglobulin are raised, especially that of IgM. IgM reached a peak early on and IgG later but there was no clear evidence of a switch from IgM to IgG production.

Wollheim and Williams (1966) showed that absorption of high titre sera with sheep cells showed no significant loss of IgM, indicating that most had no heterophil antibody activity.

Paul-Bunnell Antibody

This heterophil (PB) antibody was first found in 1932 (Paul and Bunnell 1932). In 1937 Davidsohn developed his differential absorption (PBD) test to distinguish it from Forssman antibody. The PB antibody appears to be specific for IM; Davidsohn and Lee (1969) have encountered only 2 false-positives in 30 years.

Many other tests for the PB antibody have been developed (Lee, Davidsohn and Slaby 1968, Davidsohn and Lee 1969) but the PBD test is still considered to be the best for routine diagnosis.

The PB antibody is invariably an IgM antibody, no IgG antibody having been detected (Wilkinson and Carmichael 1964, Carter 1966c). Why the switch-over does not occur is not known but in this the PB antibody is similar to the human natural anti-A and anti-B blood group antibodies (Franks and Coombs 1969).

Sera from cases of IM contain antibodies to sheep, horse, ox and rabbit red cells and to human red cells treated with Newcastle disease/

disease virus (NDV) (Wilkinson and Carmichael 1964). These workers showed that the rabbit and probably the NDV-treated human red cell antibody systems were separate from the others. These had a well-defined pattern of cross-absorption. It was suggested that these antibodies could have similar non-identical combining sites or that in IM sera there were several unrelated antibodies reacting with unrelated antigenic determinants shared by heterologous red cells. Therefore it seems the PD antibody is in fact a mixture of several different antibodies.

The receptor for the PB antibody can be removed from sheep and ox red cells by bromelin, ficin, papain, influenza virus and receptor-destroying enzyme, the effect of the last two suggesting that neuraminic acid or a derivative may be involved (Springer and Rapoport 1957, Carter 1966c). However, Lee et al (1968) found papain ineffective in reducing the titre of horse red cell agglutinins, which might mean that the structure of the horse red cell receptor is different from that on sheep and ox cells.

Two, not very satisfactory, explanations for the P+ development of the PB antibody have been reported. One is that there are cross-reacting antigens on the virus and red cells and the other that the antigen is produced by the action of a viral enzyme.

Red Cell Antibodies

Haemolytic anaemia is a rare complication of IM. This has been investigated and found to be due to anti-i antibody in a number of cases. Adult human red cells have an I antigen and a little i antigen; in ^{cord} ~~cord~~ cells the reverse is so. The antibodies/

antibodies to these antigens are cold antibodies reacting best at 4°C.

Anti-i was first found in low titre by Jenkins, Koster, Marsh and Carter (1965) in 8% of cases of IM. It was shown to be in the IgM class. Later workers found anti-i in a much higher proportion, probably because of technical differences (Rosenfield, Schmidt, Calvo and McGinniss 1965, Wollheim and Williams 1966, Kaplan 1968, Worlledge and Dacie 1969). In cases of anaemia, the antibody is still active at 28 - 30°C, although the titre may remain low (Jenkins et al 1965, Worlledge and Dacie 1969).

An IgG anti-i has now been found in 90% of IM sera (Capra, Dowling, Cook and Kunkel 1969). 66% had an IgG anti-i and cold reactive IgM rheumatoid factor mixture. It was suggested that cold agglutination was in many cases due to the interaction between these 2 antibodies. The haemolytic anaemia in their series seemed to be more related to the IgG anti-i titre than to the IgM titre; indeed two of their cases had no IgM but high titre of IgG anti-i. Mullinax, James, Mullinax and Himrod (1966) had previously suggested that IgM - IgG complexes could be responsible for haemolysis.

Anti-I may also be responsible for some cases. Kaplan (1968) found anti-I in 19 out of 21 cases and Worlledge and Dacie (1969) in a review of published cases mention 14 with raised anti-I titres. Brafeld (1966) found a cold antibody which reacted equally against I and i cells, which is probably different from anti-I/

Table 5.

Other autoantibodies in infectious mononucleosis

Rheumatoid factor (1, 2, 5, 7)

Wassermann antibody (1, 5)

Anti-nuclear factor (ANF) (1, 3, 4, 6)

Leuco-agglutinins (1)

Anti-thyroglobulin-like (1)

1. Carter 1966d.
2. Mullinax et al 1966.
3. Wollheim and Williams 1966.
4. Wager, Räsänen, Lassus and
Mustakallios 1967.
5. Kaplan 1968.
6. Kaplan and Tan 1968.
7. Capra et al 1969.

anti-I and anti-i.

The haemolysis probably occurs in the blood vessels of the skin where the temperature is low and the cold antibodies active (Worlledge and Dacie 1969). Other cells are coated with sublytic doses of complement and are removed in the normal way.

Other auto-antibodies which have been found are listed in Table 5.

Cryoglobulins

Cryoglobulins are formed in IM and precipitate out as IgM, IgG and complement mixtures (Mullinax et al 1966, Wager et al 1967, Kaplan 1968, Kaplan and Tan 1968).

Antibody activity has been detected in these complexes and the following antibodies identified: ANF, heterophil antibody, Wassermann antibody, anti-I, anti-i, and rheumatoid factor (Wager et al 1967, Kaplan 1968, Kaplan and Tan 1968, Capra et al 1969). Apart from the anti-i, these cryoglobulins are not of clinical significance in IM.

Wager et al (1967) suggested that the mixed IgM - IgG cryoprecipitate was an antigen-antibody complex. Kaplan (1968) suggested that ^{auto}~~anti~~-allergic processes may contribute to the pathogenesis of IM because of the similarity of the cryoproteins in IM to those in systemic lupus erythematosus and similar conditions.

The Allergic Reaction in IM

Why these auto-antibodies are formed is unknown. Dameshek (1969)/

(1969) has suggested that IM is an immuno-proliferative disorder like myeloma and macroglobulinaemia. This would account for the high immunoglobulin levels but not for the auto-antibodies.

One possibility is that the virus considerably increases the tendency to auto-antibody production, especially in those who are genetically predisposed to auto-antibody formation. It is suggested by Dresser and Mitchison (1968) that when tolerance is induced the antigen is presented directly to the antigen-sensitive lymphocyte and the cell is then killed. It is possible in IM that the virus makes these cells resistant to this inactivation process.

EB Virus and Infectious Mononucleosis

EBV Particles in IM

Prior to the discovery of the EB virus the only other suggested aetiological agents were Sendai virus (Evans 1960) and various ^{other} myxoviruses (Joncas, Chagnon and Pavilanis 1966, Joncas et al 1968). Attempts at experimental transmission were unsatisfactory (Evans 1960, Joncas, Lussier, Pavilanis 1966, Joncas, Chagnon, Robert and Pavilanis 1967).

A herpes-like virus, now known as the Epstein-Barr virus, was first found in cultured lymphoblasts from Burkitt's lymphoma (Epstein, Achong and Barr 1964, Epstein, Henle, Achong and Barr 1965). It was present in only 1 - 2% of cells and in 2 forms: mature/

mature particles 110 - 115 m μ diameter and smaller immature. Defective particles ~~were also seen~~. The virus was usually seen in dead and degenerating cells and less often in intact cells. It could not be isolated and identified. These findings have been abundantly confirmed (Stewart, Lovelace, Whang and Ngu 1965; Hummeler, Henle and Henle 1966; Toplin and Schidlovsky 1966).

The connection with IM was found by chance when a technician had IM and developed EBV antibodies (Henle et al 1968). The virus was then seen in her cultured lymphocytes (Diehl et al 1968).

The situation then became rather confused when Moses et al (1968) saw 22 m μ particles in all 14 cell lines studied and EBV in only 4. They suggested that the small particles could be a defective virus and the EBV the helper or that there might be 2 separate viral infections.

EB virus particles have also been seen in cultured cells derived from normal adults and patients with leukaemia and various neoplastic diseases (Zeve, Lucas, Manaker 1966; Jensen, Korol, Dittman and Medrek 1967; Moore, Serner and Franklin 1967; Pope 1968) and even in cells from cases of viral hepatitis (Glade, Hirshaut, Douglas and Hirschorn 1968). Indeed, these workers believe there is no clear relationship between the EBV and any specific disease (Glade, Hirschorn and Douglas 1969).

The reason why the EBV is seen in these various conditions is because infection with it is so common and because it remains latent in the host cells.

Epidemiology/

Epidemiology

EBV antibodies detected by complement fixing and immunofluorescence tests are very common in the general population; the incidence is high in infants, then falls off to rise again to 90% in the late teens. This seems to hold for American, British, Swedish and African populations (Levy and Henle 1966; Henle and Henle 1967; Gerber and Burch (1967); Demissie and Svedmyr (1969); Pereira, Blake and Macrae (1969)). The incidence is higher in children from poor environments (Levy and Henle 1966; Henle et al 1968) and in enclosed communities (Pereira et al 1969).

At Yale University, all IM sera were found to have EBV antibodies whilst only 24% of controls had them, reflecting the fact that Yale students come from the higher socio-economic groups. In the same study it was shown that only the EBV negative students developed IM later on (Evans et al 1968).

EBV antibodies are usually present at their peak titres on admission so it is difficult to demonstrate a rising titre (Evans et al 1968; Gerber et al 1968; Banatvala and Grylls 1969); once present they probably persist for life (Niederman, McCollum, Henle and Henle 1968).

The EBV and PB antibodies usually appear at about the same time although the EBV antibody occasionally lags behind (Niederman et al 1968). The two antibodies are quite unrelated.

Membrane antigens have been found on Burkitt lymphoma and IM cells/

cells, dependent on the presence of the EB virus. Two membrane antibodies were found in Burkitt's lymphoma but only one in IM, possibly because in IM there were too few cells with the surface antigen to keep the antibody level sufficient for detection. Usually the viral and membrane antibody levels were lower in IM than in Burkitt's lymphoma and went together but in about 20% there was a discrepancy, as yet unexplained. The membrane antigen can be detected in fresh cells and in 10 times as many as the viral antigen. To explain this, it is suggested that only 10% of infected cells can support EBV replication but in the other 90% the viral genome is not entirely dormant, as with some other tumour viruses. This account is taken from Klein et al (1968a, b, 1969) and Pearson et al (1969).

Infection and Transplantation

Henle et al (1967) successfully infected a leucocyte tissue culture with the EBV by incubating it with lethally irradiated Burkitt lymphoma cells. Stewart, Glazer, Ben and Lloyd (1968) managed to passage it in newborn thymectomised hamsters and recover and identify it in human lymphoblast cultures.

Southam, Burchenal, Clarkson, Tanzi, Mackey and McComb (1969) transplanted IM cells into newborn rats in which they produced "extensive tumorous growths". The significance is difficult to evaluate since cells from an apparently normal lymph node did the same. These workers suggested that malignant transformation occurred in culture as has happened with other cell lines.

Evans/

Evans, Niederman and McCollum (1969) allude to a report of the injection of EBV causing an IM-like illness in a leukaemic patient. This ^{may be} ~~is~~ the first successful transmission of EBV giving rise to IM.

Properties of Infected Cells

Leucocytes from IM and other conditions are readily established in long-term culture (Diehl et al 1968, Glade et al 1968b, Henle et al 1968). All these cultures contained the EB virus; it has even been suggested that the EB virus may be responsible for the long-term proliferative potential of these cells (Henle, Diehl, Kohn, Zur Hausen and Henle 1967).

These cultured lymphoblasts are very pleomorphic (Glade et al 1968b) but there is some doubt about their relationship to the AMC of the peripheral blood (Glade and Chessin 1968) because of some of their properties: they can synthesise immunoglobulins (Glade and Chessin 1968); contain virus particles; have a karyotypic abnormality in a group C chromosome (Kohn, Diehl, Mellman, Henle and Henle 1968). Whether this marker is an artefact or represents oncogenic potential or is a marker of latent viral infection is not known (Chessin, Glade, Kasel, Moses, Herberman and Hirshaut 1968). They also produce interferon when cellular, and presumably viral, proliferation occurs (Kasel, Haase, Glade and Chessin 1968). These properties are shared by Burkitt lymphoma cells. There is now some evidence consistent with a clonal/

clonal origin of Burkitt's lymphoma (Fialkow, Klein, Gartler and Clifford 1970); it would be very interesting to see if IM^{cell} also have a clonal origin.

EB Virus Antigens

In addition to the complement fixing antibody to the EB virus (Gerber et al 1968) immunofluorescent techniques have revealed others.

Using fixed cells a viral antigen could be detected in 6% of the lymphoblasts (Diehl et al 1968). It was suggested that its absence in fresh cells was due to a carry-over of blocking antibodies but it seems more likely to be due to the latency of the virus in vivo, like other herpes viruses, the in vitro conditions being necessary for its replication.

Association of the EB Virus with Burkitt's Lymphoma and Infectious Mononucleosis

Any theory linking the EBV and disease has to explain its connection with these 2 apparently very different disorders. There seems to be a connection with IM but it is certainly not a "straightforward and simple" aetiological one (McCollum, Niederman, Evans and Giles 1969).

Three possible roles have been suggested for the virus: it could be the direct cause; or a latent virus activated by lymphoreticular proliferation; or a super-infection (Chessin et al 1968, Lancet 1969, Carter 1969b). Although none of these can be ruled out, the indirect roles seem less likely because of the sero-epidemiological/

sero-epidemiological evidence.

However, Burkitt's lymphoma and IM are not so different as at first glance. The properties of their cultured cells are very similar, although some of them could be artefacts of tissue culture. Both have been transplanted into rats. The Henles (See Lancet 1969) have reported a case of Burkitt's lymphoma 6 months after an attack of IM.

Burkitt (1969) has put forward a very ingenious hypothesis to link the two conditions. The distribution of Burkitt's lymphoma matches that of holoendemic malaria (Daldorf, Linsell, Barnhart and Martyn 1964), which is an obvious cause of sustained lympho-reticular stimulation. The hypothesis is as follows:

1. Throughout the world the EBV normally induces a subclinical infection but occasionally gives rise to a non-malignant lymphoid proliferation in IM.
2. In the absence of chronic stimulation it may very rarely initiate malignant lymphoid proliferation in isolated single cases of Burkitt's lymphoma.
3. When interacting with chronically stimulated lymphoid tissue, malignant transformation may occur in a higher proportion of cases.

Further study of IM will help us to understand this disease and its relationship with the EBV and Burkitt's lymphoma and possibly with neoplastic processes in lymphoid tissues.

Conclusions

1./

Conclusions

1. IM is a relatively mild illness with limited signs and symptoms.
2. It is probably transmitted by close, and possibly intimate, contact. Its incubation period is 5 - 6 weeks. Large epidemics probably do not occur.
3. The AMC probably derives from the lymphoid series but does not produce antibody. It is a very active cell and has a high rate of division. Its origin and fate are still uncertain but it may end up as a small round cell. It has similarities with leukaemic cells but there are important differences.
4. Why the Paul-Bunnell antibody appears is still unknown. It probably consists of several different heterophil antibodies. It is also not known why the auto-antibodies appear.
5. The EB virus is closely linked with both Burkitt's lymphoma and infectious mononucleosis. Sub-clinical infection appears to be common in children. How these two, apparently very different, diseases are connected is as yet uncertain although a very ingenious theory has been put forward.

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